

Relationships among body size components of three flightless New Zealand grasshopper species (Orthoptera, Acrididae) and their ecological applications

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Abstract

Body size is perhaps the most fundamental property of an organism and is central to ecology at multiple scales, yet obtaining accurate estimates of ecologically meaningful size metrics, such as body mass, is often impractical. Allometric scaling and mass-to-mass relationships have been used as alternative approaches to model the expected body mass of many species. However, models for predicting body size in key herbivorous insects, such as grasshoppers, exist only at the family level. To address this data gap, we collected empirical body size data (hind femur length and width, pronotum length, live fresh mass, ethanol-preserved mass, and dry mass) from 368 adult grasshoppers of three flightless species at Hamilton Peak, Southern Alps, New Zealand. We examined the relationships among body size components across all species using linear and non-linear regression models. Femur length and preserved mass were robust predictors of both fresh mass and dry mass across all species; however, regressions using preserved mass as a predictor always showed higher predictive power than those using femur length. Based on our results, we developed species-specific statistical linear mixed-effects models to estimate the fresh and dry masses of individual grasshoppers from their preserved mass and femur length. Including sex as an additional co-variate increased model fit in some cases but did not produce better estimates than traditional mass-to-mass and allometric scaling regressions. Overall, our results showed that two easy-to-measure, unambiguous, highly repeatable, and non-destructive size measures (i.e., preserved mass and femur length) can predict, to an informative level of accuracy, fresh and dry body mass across three flightless grasshopper species. Knowledge about the relationships between body dimensions and body mass estimates in these grasshoppers has several important ecological applications, which are discussed.

Keywords

allometric scaling, body mass, linear body dimension, mass-to-mass relationships, predictive models

Introduction

Organism body size is one of the most important axes in ecology, as it is related to nearly all biological processes, from individual performance to ecosystem function (Whitman 2008, Chown and Gaston 2010). In insects, body size is closely linked to

physiological rates (e.g., metabolic and growth), life-history traits (e.g., longevity and fecundity), and ecological attributes, such as abundance, range size, and dispersal (Peters 1983, Siemann et al. 1996, Whitman 2008, Chown and Gaston 2010, Ehnes et al. 2011, Stevens et al. 2012). Moreover, arthropod body size is central to the contribution of individuals and communities to key ecosystem processes and services, such as decomposition, carbon cycling, primary productivity, pollination, predation, and herbivory (Cízek 2005, Barnes et al. 2018, Kendall et al. 2019). Therefore, changes in the body size of a taxon reflect changes in resources that may cascade across all levels of biological organization. For example, body size differences are usually associated with individual survival and fecundity, and changes in body size might alter ecological processes, including trophic interactions, plant–animal interactions, and food web connectivity (Peters 1983, Stang et al. 2009, DeLong et al. 2015, Horne et al. 2018).

Adult body size in Orthoptera is generally expressed in terms of length and mass, each of which is controlled by both genetic and environmental factors that operate through molecular and physiological mechanisms (Nijhout 2003, Whitman 2008, Chown and Gaston 2010). Although length and mass are often correlated, each captures a different aspect of an organism's size and is subject to different selective pressures during an organism's lifespan (Gaston and Blackburn 2000). Insect structural body size (e.g., length dimensions) is determined during development by gene–environment interactions, whereas adult body mass additionally varies through time depending on environmental factors, for example, reproductive phase and nutritional status (Whitman 2008, Chown and Gaston 2010, Knapp and Knappová 2013). Despite this fact, body mass- and linear-based estimates are often used interchangeably as measures of adult body size in ecological research (Chown and Gaston 2010). Decisions on the body size measure used in a particular study should be made cautiously and considering the research question and species (Gaston and Blackburn 2000, Moretti et al. 2017).

Body mass is the most meaningful size metric, as it is directly linked with metabolic rate and is affected by environmental conditions (Gaston and Blackburn 2000, Sohlström et al. 2018). Therefore, fresh (live) mass is preferred to relate body size to a range

of functional and ecological attributes, such as metabolism, movement, and abundance (e.g., Chown and Steenkamp 1996, Meehan 2006, Ehnes et al. 2011, Hirt et al. 2017). In some instances, however, dry mass is recorded to estimate, for example, organism biomass, since variation from water content is reduced (e.g., Sage 1982, Cressa 1999, Sabo et al. 2002, Gilbert 2011, Penell et al. 2018). While body mass is a useful predictive trait for many ecosystem processes, measuring individual arthropod body mass is a time-consuming and tedious process (Johnston and Cunjak 1999, Eklöf et al. 2017, Sohlström et al. 2018, Kendall et al. 2019). Moreover, collection and storage methods often prevent the direct determination of mass estimates, especially when specimens are damaged (e.g., loss of appendages) or when subject to chemical preservation that causes unpredictable mass change (Johnston and Cunjak 1999, Wetzel et al. 2005, Chown and Gaston 2010, Moretti et al. 2017). As a result, most ecological studies on insects rely on more easily measured body dimensions (e.g., body length) as proxies for body size (Chown and Gaston 2010). Many insect collections are composed of specimens preserved in ethanol, and these collections provide an important source of information about organismal change over time if we can convert preserved mass to biologically meaningful measures.

Allometric scaling rules applied to co-varying traits can be used to predict an organism's body mass based on an easy-to-obtain body length measurement, thus avoiding the use of problematic body mass estimators (Johnston and Cunjak 1999, Moretti et al. 2017, Pennell et al. 2018, Kendall et al. 2019). Scaling equations have proven to be powerful tools for the prediction of body mass for a wide range of insect taxa based on different linear metrics (e.g., Rogers et al. 1977, Schoener 1980, Johnston and Cunjak 1999, Sabo et al. 2002, García-Barros 2015, Kendall et al. 2019). These equations rely on regression parameters estimated for length–mass relationships, which are often subject to intersexual allometric differences (Hagen and Dupont 2013, Kendall et al. 2019). Incorporating sexual size dimorphism data into scaling relationships, and thus their regression parameters, is crucial to overcome this limitation (e.g., Kendall et al. 2019). Despite the broad application of allometric scaling in ecological research, there are surprisingly few studies providing regression parameters for estimating the body mass of key herbivorous taxa, such as grasshoppers (but see Schoener 1980, Sabo et al. 2002 for allometric equations at the ordinal level).

Short-horn grasshoppers (Orthoptera: Acrididae) are among the most diverse (> 6,700 described species) and ubiquitous fauna of grassland ecosystems around the world (Uvarov 1966, Latchininsky et al. 2011, Song et al. 2018) contributing, in some cases, to more than half of the total above-ground arthropod biomass (Gillon 1983, Song et al. 2018). The endemic short-horn grasshoppers of Aotearoa New Zealand occur widely, but are especially abundant in alpine habitats (Bigelow 1967, Trewick 2001, Trewick 2008, Trewick and Morris 2008, Koot et al. 2020). As major invertebrate herbivores in native grassland ecosystems (Batcheler 1967, White 1975), these grasshoppers might play a major role in structuring plant communities and regulating ecosystem function via plant productivity, competition, and nutrient cycling (Olff and Ritchie 1998, Belovsky and Slade 2000, Moretti et al. 2013, Deraison et al. 2015). Given the ecological importance of grasshoppers, the determination of allometric scaling relationships provides an opportunity to explore ecologically important traits and variations that are otherwise difficult to measure.

Body size data have been accumulated for New Zealand grasshoppers mostly as linear dimensions: hind femur length and width, and pronotum length (e.g., Batcheler 1967, Staples 1967,

Bigelow 1967, Mason 1971; but see Dowle et al. 2014, Carmelet-Rescan et al. 2021). However, the suitability of these measures as predictors of body size and their relationship with other body mass estimates have not been tested. A key feature of grasshoppers is the use of jumping in locomotion and predator avoidance (Queathem 1991), and this is especially true for flightless species such as those found in New Zealand. Therefore, the size of the hind jumping leg may be closely related to other size components and, thus, to overall body size. The marked sexual size dimorphism of most grasshoppers might compound intraspecific differences in the relationships among body size components. Here, we examined these relationships focusing on three brachypterous and flightless species of the endemic alpine radiation of Kā Tiritiri-o-te-moana, the Southern Alps (Bigelow 1967, Trewick and Morris 2008, Koot et al. 2020; Fig. 1A–C): *Brachaspis nivalis* (Hutton, 1987), *Paprides nitidus* Hutton, 1987, and *Sigaust australis* (Hutton, 1987). First, we quantified the effects of short-term ethanol preservation by describing the weight change over 120 days. Then, we examined scaling ratios to assess the predictive power of preserved mass for both fresh and dry masses. We also analyzed intraspecific length–mass relationships over an elevation gradient to account, at least partially, for environmental variation in body size. Based on our results, we developed species-specific statistical models to estimate the fresh and dry mass of individual grasshoppers from their preserved mass and hind femur length. Overall, our models showed high predictive power such that body mass estimates derived from them can be used to test mechanistic hypotheses for shifts in morphological and ecological traits related to body size.

Materials and methods

Specimen collection and measurements.—A total of 368 complete adult specimens (no missing appendages) representing three grasshopper species (*B. nivalis* 61♂, 71♀; *P. nitidus* 73♂, 73♀; *S. australis* 42♂, 48♀) were collected on Hamilton Peak in the Craigieburn Range, New Zealand (−43.129, 171.688; WGS84). Sampling was done by hand, capturing grasshoppers disturbed by walking at five sites at ~100 m elevation intervals (BR1 to BR5) from 1,383 to 1,817 m asl, to capture as much local variation in body size as possible. Species and sex were recorded from live specimens in the field and were later corroborated upon processing based on morphological features (e.g., body color pattern, pronotum shape, and body shape and size) following Bigelow (1967). Maturity and sex were determined using the size and shape of the tegmina and terminalia (Bigelow 1967).

Grasshoppers were weighed alive after cooling to 4 °C, then frozen overnight before being preserved in 95% ethanol for DNA preservation. Specimens were weighed using a Sartorius Quintix35–1S digital scale (Sartorius Lab Instruments GmbH & Co, Goettingen, Germany) accurate to 0.001 g. We measured the left hind femur length (hereafter femur length) and width (hereafter femur width), and pronotum length of specimens (Fig. 1D) using an Olympus SZX7 stereomicroscope with Olympus SC100 image capture and Olympus cellSens Dimension v1.6 software (Olympus Corporation, Tokyo, Japan). These measures were chosen because they are commonly used proxies for body size in grasshoppers (e.g., Bigelow 1967, Mason 1971, Harris et al. 2012, Yadav et al. 2018).

To quantify the effects of our preservation method on body mass estimates, we remeasured the body mass of all specimens after two and four months of storage in ethanol. Once all other measurements were completed, a random subsample of 50 specimens of each species (25 males and 25 females) were dried in an

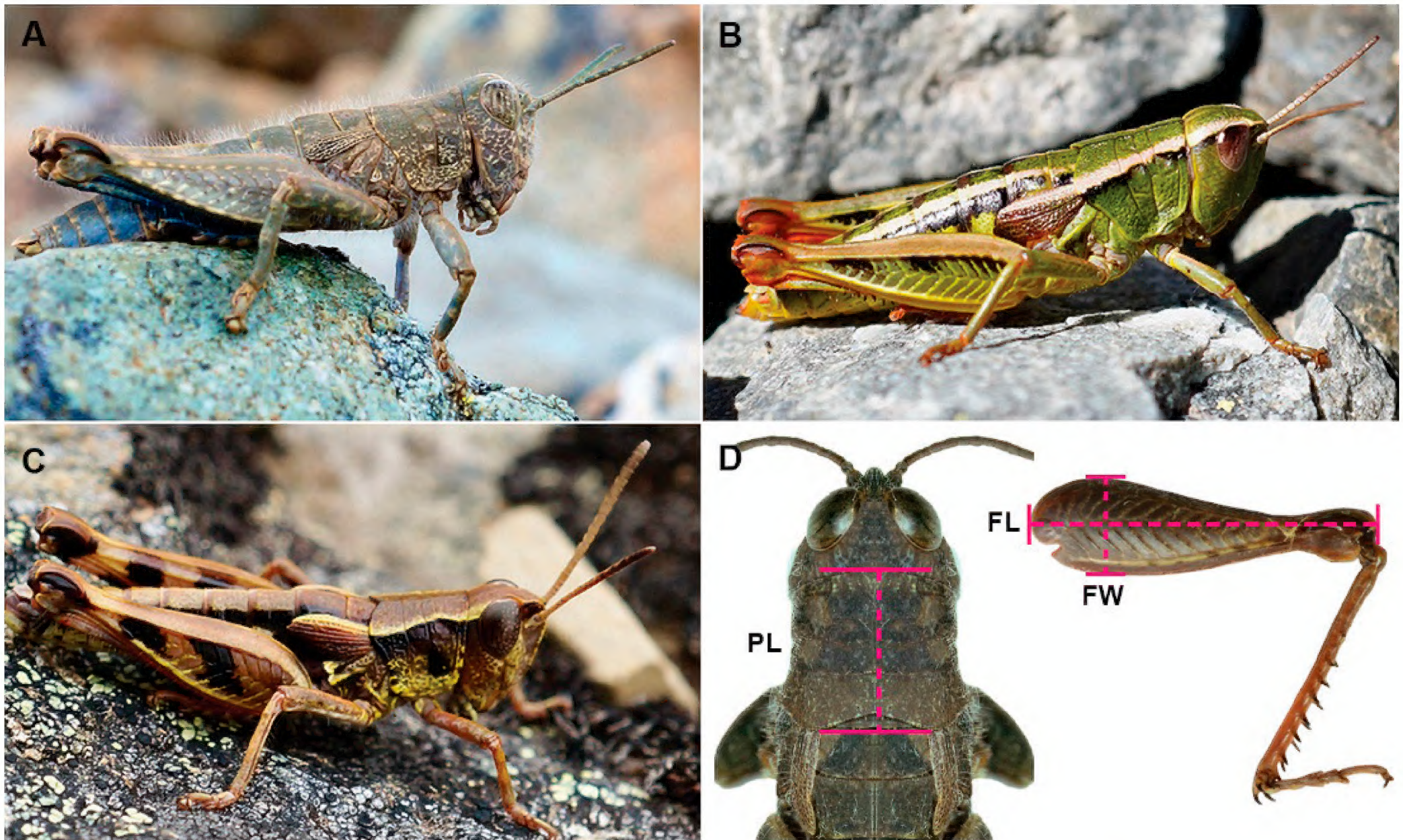


Fig. 1. Adult endemic, brachypterous, and flightless grasshopper species from Hamilton Peak in the Southern Alps, New Zealand. A. *Brachaspis nivalis* female; B. *Paprides nitidus* female; C. *Sigauss australis* male; D. Body dimensions used as proxies of overall body size in this study: morphometric data were collected for hind femur length (FL), hind femur width (FW), and pronotum length (PL).

oven at 60°C for at least 96 h, until their mass ceased to change, and were then weighed. To assess measurement repeatability, we randomly selected five males and five females of each species and remeasured and reweighed them three times in random order.

Data analysis and model structures.—Repeatability (R) was calculated independently for species and sexes with the R package rptR (Stoffel et al. 2017), using specimen as a grouping term. The ratio of intra-observer variance (i.e., R) was calculated as the among-group variance (VG) over the sum of group-level and within-group (residual) variance (VR): $R = VG / (VG + VR)$. Confidence intervals (95%) around repeatability values were estimated using 1,000 parametric bootstrap iterations. The effect of preservation in 95% ethanol on specimen body mass was examined by comparing the mass of individuals when live (fresh mass) and after ethanol preservation for two and four months. We also examined the frequency distributions of differences in body mass before and after preservation for each species. As the shape of the size–frequency distribution was almost identical for both preserved states (Fig. 2), we used a Wilcoxon signed-rank test to analyze overall and sex-specific differences between fresh mass and preserved mass after four months of preservation (hereafter preserved mass), pooling data from all species. For these analyses, a non-parametric approach was preferred, as mass difference between live and 4-month preserved specimens was not normally distributed when considered together. Statistical tests were implemented using the R package rstatix version 0.7.0 (Kassambara 2021).

We explored mass-to-mass ratios between ethanol preserved mass (after four months of preservation, PM), and both fresh mass

(FM) and dry mass (DM) for each species, using model II regressions with standardized major axis (SMA) in the R package smatr version 3.4-8 (Warton et al. 2012). We performed SMA regressions by (i) including an intercept term (i.e., not forced through the origin) under the robust outlier option and (ii) assuming that changes in any body mass metric is reflected in the other metric, as measurements came from the same specimens ($y = 0$ when $x = 0$), and forcing the intercept through the origin (i.e., zero-intercept). We also tested for a common slope between sexes and among sites (i.e., elevation) with an ANCOVA-like test, using the slopes estimated in SMA regressions (Warton et al. 2012). Since preserved mass was closely related to the other measures of mass ($R^2 \geq 0.913$, $p < 0.001$; for additional details see Results), we specified a series of species-specific linear mixed-effects (LMM) models to predict FM and DM as a function of PM using the R package lme4 version 1.1-27.1 (Bates et al. 2015). This approach allowed us to account for sex- and site-specific differences in body mass by including sex as an additional fixed effect and as an interaction term with preserved mass, elevation as a random intercept, and preserved mass as a random slope.

We used ordinary least squares (OLS) regressions in R base (R Core Team 2020) to compare body dimensions (femur length = FL, femur width = FW, and pronotum length = PL) as predictors of body mass components (i.e., FM and DM) using log-transformed data. For each species, we estimated and compared the slopes of fitted lines between sexes using the R package emmeans version 1.6.2-1 (Lenth 2021). As the strength of relationships varied between sexes and in some instances presented apparent deviations from linearity (see Results), we fitted sex-specific non-linear mod-

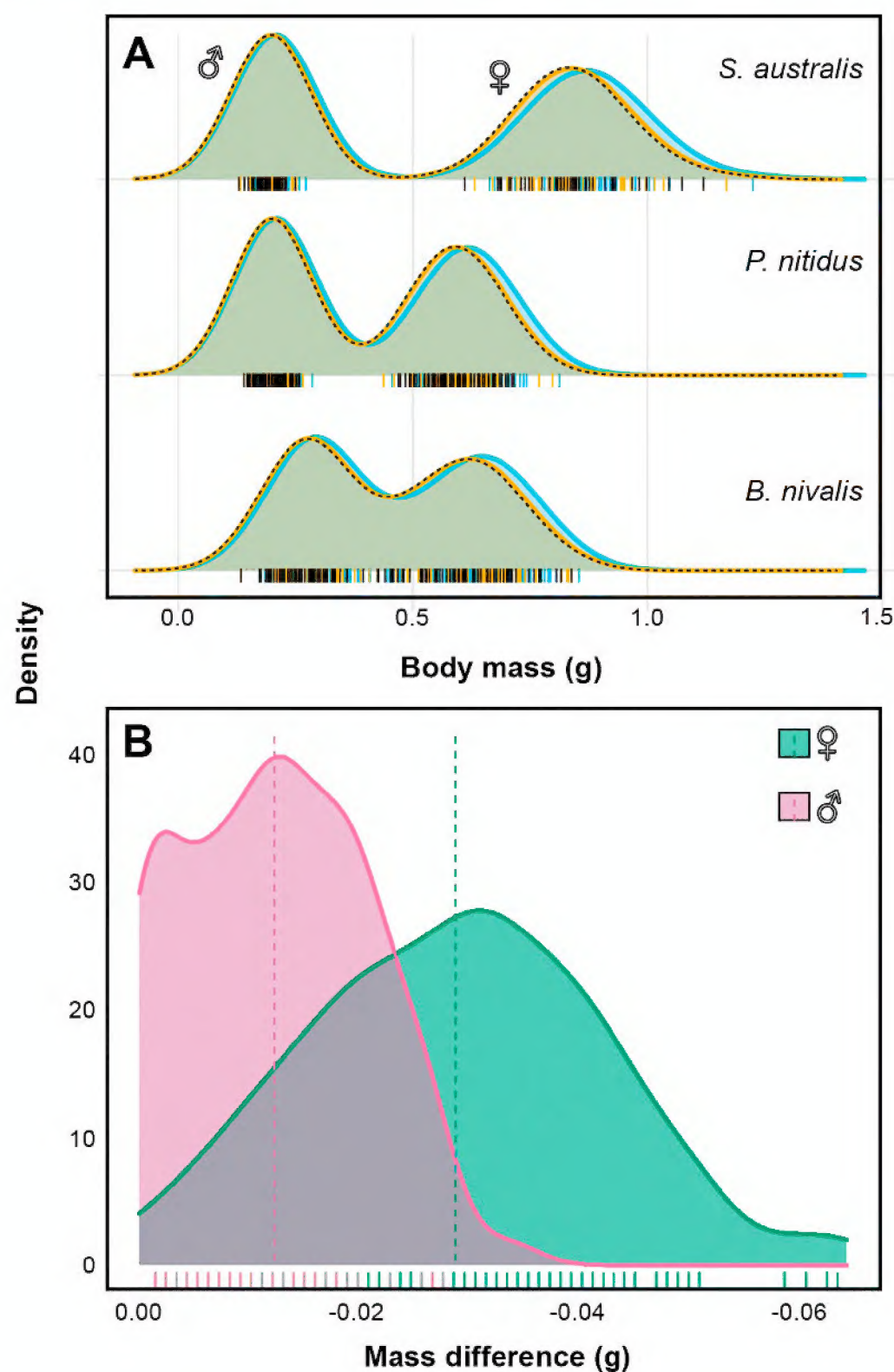


Fig. 2. A. Density distributions of body mass in three flightless New Zealand grasshopper species when alive (turquoise) and after ethanol-preservation for two (dark yellow) and four months (black); B. The distribution of the difference in mass between live and 4-month preserved specimens pooled for all three species and partitioned by sex. Mean values for male (-0.012 g) and females (-0.029 g) are indicated by dashed lines. Marginal rug indicates individual observations of body mass.

els (Knell 2009) to analyze the shape of the scaling relationship. Five models were compared using Akaike's Information Criteria (AIC): (i) quadratic, (ii) logistic, (iii) four-parameter logistic, (iv) Weibull growth function, and (v) power function models. Models were fitted on untransformed variables (Packard 2011) using base R (R Core Team 2020) and the R package *aomisc* version 0.647 (Onofri 2020). We chose femur length for the following analyses because it was highly correlated with all other body dimensions (Pearson's $R > 0.924$, $p < 0.001$) and easier to measure consistently, as indicated by our repeatability analysis (Suppl. material 1: Appendix 1).

We further explored scaling relationships between FL and both FM and DM using model II regressions SMA including only an intercept term (i.e., not forced through the origin), as the femur length of adult insects does not change in response to changes in body mass (Whitman 2008, Chown and Gaston 2010, Bailey et al. 2020). We also specified LMMs using FL as a predictor of both FM and DM, using homologous model structures as defined

previously for mass-to-mass modeling, to account for sex- and site-specific differences in trait variability. These approaches were chosen because sex-specific linear models generally performed as well as or better than non-linear models ($\Delta AIC \leq 1.95$), although when predicting dry mass for females of *B. nivalis*, the quadratic model performed slightly better than the linear model ($\Delta AIC = 2.61$). For model formulation, we used log-transformed values because static allometric relationships explored here are generally well-described by a power function ($y = ax^b$), which is linearized when log-transformed: $\ln(y) = \ln(a) + \beta \times \ln(x) + e$, where y = dry mass, a = intercept, β = allometric coefficient, and x = linear size proxy.

The best-fitted models (both allometric and LMMs) were selected using Akaike's information criterion corrected for sample size (AICc) and Akaike weight (w_i) using the R package *AICcmodavg* version 2.3-1 (Mazerolle 2020). Models with $\Delta AICc < 2$ were considered equally supported by the data, while models with $\Delta AICc > 2$ were considered to show substantial differences (Burnham and Anderson 2002). The Akaike weight (w_i) was interpreted as the probability that model i was the best model given all evaluated models and data available (Burnham and Anderson 2002). For all models, the goodness of fit was examined by calculating conditional R^2 using the R package *MUMIn* version 1.43.17 (Barton 2020). The statistical significance of fixed and random effects was examined for the best-fitted models using the R package *lmerTest* version 3.1-3 (Kuznetsova et al. 2017). Assumptions of model fit were met for all models as indicated by diagnostic plots of residuals.

Testing model accuracy.—We predicted fresh and dry body mass for 368 grasshopper specimens using mass-to-mass ratios, scaling regressions, and parameters from the best-fitted LMMs. We then tested the relationship between measured and predicted values using model II regressions with a major axis approach using the R package *lmodel2* version 1.7-3 (Legendre 2018). This method is appropriate when comparing empirical observations to model predictions (Legendre and Legendre 2012). The statistical significance of relationships was tested using one-tailed permutation tests (with 1,000 permutations), and the strengths of the relationships were determined by model R^2 values. Observed relationships were also compared to the ideal $x = y$ association where estimated = measured by calculation of 95% confidence intervals around the estimated slope. The accuracy of our predictions was also estimated using the root-mean-square error (RMSE) between the observed and predicted values, using the R package *Metrics* version 0.1.4 (Hamner and Frasco 2018). All analyses were performed using R 4.0.3 (R Core Team 2020).

Results

We found high measurement consistency ($R > 0.970$), although the degree of repeatability differed among body size proxies, species, and sexes, reflecting the relative size of the values (Suppl. material 1: Appendix 1). The highest mean repeatability was recorded for the larger traits (femur length $R = 0.9990 \pm 0.0001$ SD, preserved mass $R = 0.9985 \pm 0.0001$ SD), the larger species (*B. nivalis* $R = 0.9941 \pm 0.0082$ SD and *S. australis* $R = 0.9941 \pm 0.0094$ SD compared to *P. nitidus* $R = 0.9912 \pm 0.0147$ SD), and the larger sex (females $R = 0.9953 \pm 0.0068$ SD compared to males $R = 0.9907 \pm 0.0148$ SD). Overall, grasshopper specimens weighed significantly less after four months in ethanol than when they were alive (Wilcoxon's test $p < 0.001$; Fig. 2A), although differences were small ($4.606\% \pm 2.705$ SD). On average, the larger female specimens

lost more weight than the male specimens (Wilcoxon's test $p < 0.001$; Fig. 2B; see Suppl. material 1: Appendix 2 for species details).

There were strong and significant relationships between preserved mass (PM) and both fresh mass (FM, $R^2 \geq 0.997$, $p < 0.001$) and dry mass (DM, $R^2 \geq 0.913$, $p < 0.001$) in all species (Fig. 3; Suppl. material 1: Appendix 3). No significant differences in slopes were indicated by the ANCOVA-like test for the two sexes, but site differences were found when predicting DM as a function of PM in *S. australis* (Suppl. material 1: Appendix 3). Estimated ratios of preserved to fresh mass (mean ratio = 1.041 ± 0.005 SD) and preserved to dry mass (mean = 0.310 ± 0.008 SD) were similar for all species (Table 1). All LMMs including co-variables exhibited similar overall predictive power as judged by their fitting scores (Table 2). When predicting fresh mass as a function of preserved mass, the PM-only fixed-effect model incorporating site as a random effect (FM~PM+(1|Site)) outperformed other models for all species, except *B. nivalis* (Table 2a). For this species, one of the models accounting for sexual dimorphism exceeded the baseline model (i.e., FM~PM+(1|Site)) in terms of AICc ($\Delta\text{AICc} = 3.47$, $\Delta\text{wi} = 0.54$) but not R^2 ($\Delta R^2 = 0.001$). In contrast, when predicting dry mass, one of the models accounting for sexual dimorphism and site differences (FM~PM+Sex+(PM|Site)) surpassed other models for all species (Table 1b) except *B. nivalis*. In this species, the PM-only fixed-effect model outperformed models including sex in terms of AICc ($\Delta\text{AICc} = 2.47$, $\Delta\text{wi} = 0.51$) but not R^2 ($\Delta R^2 = 0.000$). Fixed effects were significant in all best-fitted models ($p > 0.001$), yet

the random effect (i.e., site) was only significant when predicting FM for *S. australis* ($p > 0.001$; Suppl. material 1: Appendix 4). All LMMs outperformed the null models (i.e., FM~1+(1|Site) and DM~1+(1|Site)) in their predictive power (Table 2).

As expected, there was a strong and significant correlation (Pearson's $R \leq 0.893$, $p < 0.001$) among all body size measures, with pairwise comparisons involving femur length (FL) having the highest correlation coefficients (Pearson's $R > 0.924$, $p < 0.001$; Suppl. material 1: Appendix 5). All body dimensions exhibited strong and significant linear relationships with both fresh mass ($R^2 \geq 0.938$, $p < 0.001$) and dry mass ($R^2 \geq 0.887$, $p < 0.001$), although the strength of these relationships differed between sexes and, in some cases, appeared nonlinear (Suppl. material 1: Appendix 5). Differences in slopes between sexes were subtle for all species, and a significant difference was only detected when predicting FM in the function of pronotum length (PL) for *S. australis* ($p = 0.007$, Suppl. material 1: Appendix 5). Comparisons of sex-specific models showed that, in most cases, linear models performed as well as or better than alternative non-linear models. However, slight deviation from linearity was detected when predicting DM for female *B. nivalis*, where an allometric quadratic model performed marginally better than a linear model for females ($\Delta\text{AIC} = 2.61$), although both models were comparable for males ($\Delta\text{AIC} = 1.88$). Scaling relationships between body mass estimates and femur length were generally well-described by a power function (Suppl. material 1: Appendix 6). The coefficients from SMA regressions were similar for all species

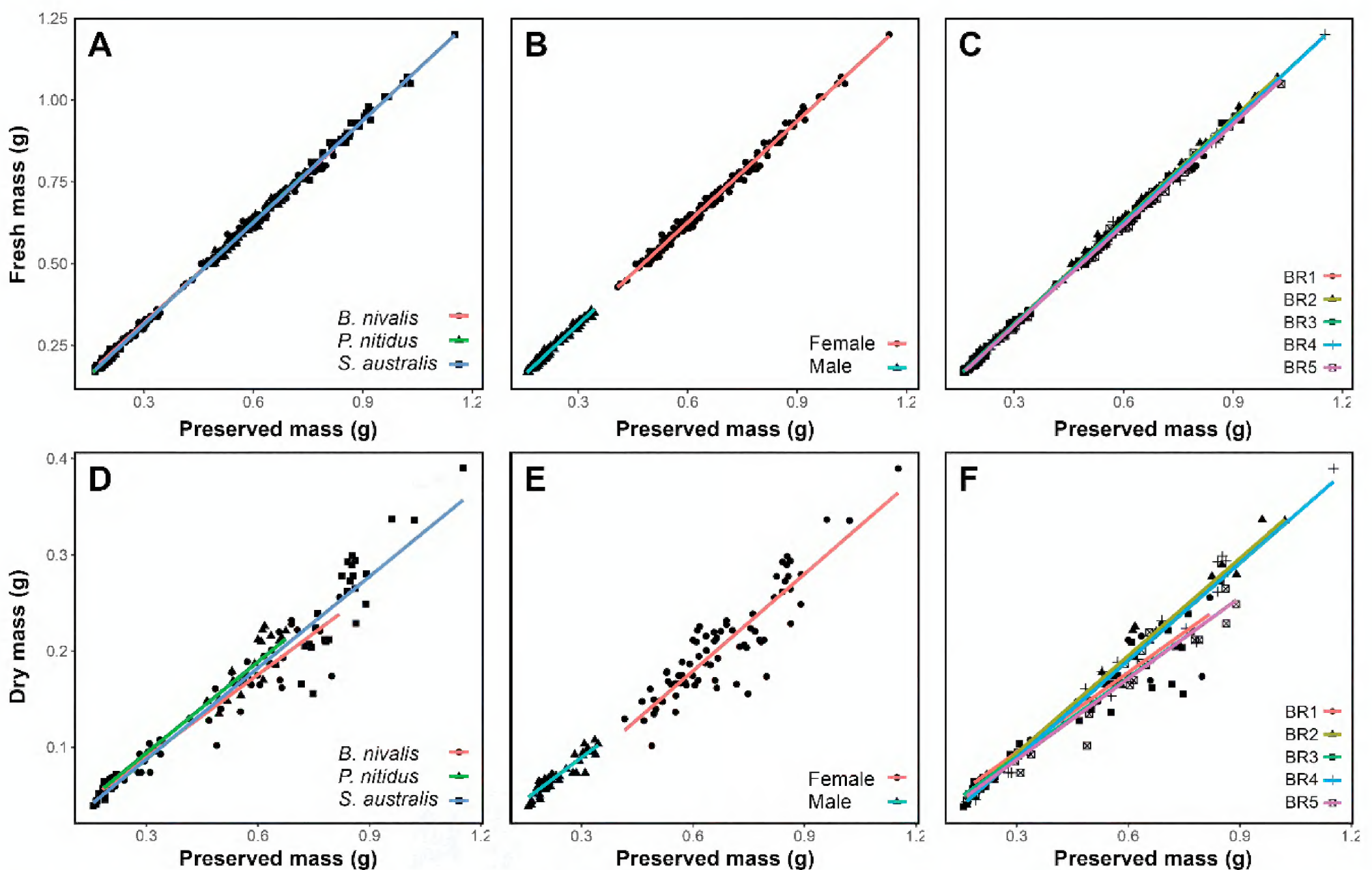


Fig. 3. Mass-to-mass relationships in three flightless New Zealand grasshopper species showing the influence of elevation and sexual dimorphism. Fresh mass–preserved mass (A–C) and dry mass–preserved mass (D–F). Sample sites (BR1 to BR5) indicating five sites in ~100-m elevation intervals from 1,383 to 1,817 m asl. Lines represent the best-fit from standardized major axis regressions. Credible intervals are omitted for clarity. Some regression lines overlaid each other.

Table 1. Mass-to-mass ratios for predicting both fresh and dry mass from preserved mass in three flightless New Zealand grasshopper species. Regression parameters based on standardized major axis regressions and their confidence intervals (95% CI) are shown.

Species	SMA	Intercept _(CI)	Slope _(CI)	R ²	p-value	Ratio
(a) Preserved mass to fresh mass (PM:FM)						
<i>Brachaspis nivalis</i>	0-intercept	0.000	1.045 _(1.041, 1.050)	0.999	< 0.001	FM=1.045PM
<i>Brachaspis nivalis</i>	intercept	0.009 _(0.004, 0.013)	1.030 _(1.021, 1.039)	0.997	< 0.001	FM=1.030PM
<i>Paprides nitidus</i>	0-intercept	0.000	1.045 _(1.041, 1.049)	0.999	< 0.001	FM=1.045PM
<i>Paprides nitidus</i>	intercept	0.000 _(-0.003, 0.003)	1.045 _(1.038, 1.052)	0.998	< 0.001	FM=1.045PM
<i>Sigauss australis</i>	0-intercept	0.000	1.042 _(1.038, 1.045)	0.999	< 0.001	FM=1.042PM
<i>Sigauss australis</i>	intercept	0.002 _(-0.001, 0.006)	1.039 _(1.033, 1.045)	0.998	< 0.001	FM=1.039PM
(b) Preserved mass to dry mass (PM:DM)						
<i>Brachaspis nivalis</i>	0-intercept	0.000	0.296 _(0.286, 0.306)	0.986	< 0.001	DM=0.296PM
<i>Brachaspis nivalis</i>	intercept	-0.004 _(-0.014, 0.005)	0.308 _(0.289, 0.330)	0.913	< 0.001	DM=0.308PM
<i>Paprides nitidus</i>	0-intercept	0.000	0.316 _(0.308, 0.323)	0.993	< 0.001	DM=0.316PM
<i>Paprides nitidus</i>	intercept	0.001 _(-0.004, 0.006)	0.310 _(0.297, 0.324)	0.969	< 0.001	DM=0.310PM
<i>Sigauss australis</i>	0-intercept	0.000	0.308 _(0.298, 0.319)	0.987	< 0.001	DM=0.308PM
<i>Sigauss australis</i>	intercept	-0.009 _(-0.019, 0.001)	0.321 _(0.304, 0.338)	0.959	< 0.001	DM=0.321PM

Table 2. Model selection showing the best-fitted models (AICc in bold) for predicting both fresh mass and dry mass from preserved mass in three New Zealand flightless grasshopper species. Abbreviations: K = number of parameters, AICc = Akaike's information criterion corrected for sample size, wi = Akaike weight, LL = Log-Likelihood, R² = marginal R². Model parameters of the best-fitting models (Δ AICc < 2) used for predictions are shown in Suppl. material 1: Appendix 4.

Species	Model formulae	K	AICc	Δ AICc	wi	LL	R ²
(a) fresh mass (FM) as a function of preserved mass (PM)							
<i>Brachaspis nivalis</i>	FM~PM+Sex+(1 Site)	5	-808.58	0.00	0.66	409.53	0.997
	FM~PM+Sex+(PM Site)	7	-807.01	1.57	0.23	410.96	0.997
	FM~PM*Sex+(1 Site)	6	-806.38	2.20	0.22	409.53	0.997
	FM~PM+(1 Site)	4	-805.11	3.47	0.12	406.71	0.996
	FM~1+(1 Site)	3	-64.11	744.47	0.00	35.15	0.112
<i>Paprides nitidus</i>	FM~PM+(1 Site)	4	-937.02	0.00	0.67	472.65	0.998
	FM~PM+Sex+(1 Site)	5	-934.87	2.14	0.23	472.65	0.998
	FM~PM*Sex+(1 Site)	6	-933.26	3.75	0.10	472.93	0.998
	FM~PM+Sex+(PM Site)	7	-931.53	5.49	0.04	473.17	0.998
	FM~1+(1 Site)	3	-35.70	901.31	0.00	20.94	0.000
<i>Sigauss australis</i>	FM~PM+(1 Site)	4	-566.34	0.00	0.50	288.35	0.999
	FM~PM+Sex+(1 Site)	5	-565.69	0.65	0.36	287.41	0.999
	FM~PM*Sex+(1 Site)	6	-563.69	2.65	0.13	288.20	0.999
	FM~PM+Sex+(PM Site)	7	-561.04	5.31	0.03	288.20	0.999
	FM~1+(1 Site)	3	69.40	635.75	0.00	-31.56	0.000
(b) dry mass (DM) as a function of preserved mass (PM)							
<i>Brachaspis nivalis</i>	DM~PM+(1 Site)	4	-259.16	0.00	0.73	134.03	0.915
	DM~PM+Sex+(1 Site)	5	-256.70	2.47	0.21	134.03	0.915
	DM~PM*Sex+(1 Site)	6	-254.14	5.02	0.06	134.05	0.915
	DM~PM+Sex+(PM Site)	7	-251.39	7.77	0.01	134.03	0.915
	DM~1+(1 Site)	3	-139.34	119.82	0.00	72.93	0.000
<i>Paprides nitidus</i>	DM~PM+Sex+(PM Site)	7	-300.56	0.00	0.67	158.65	0.981
	DM~PM+Sex+(1 Site)	5	-298.31	2.25	0.22	154.85	0.976
	DM~PM*Sex+(1 Site)	6	-296.03	4.53	0.07	155.02	0.976
	DM~PM+(1 Site)	4	-294.75	5.81	0.04	151.83	0.972
	DM~1+(1 Site)	3	-125.73	174.83	0.00	66.13	0.000
<i>Sigauss australis</i>	DM~PM+Sex+(PM Site)	7	-258.46	0.00	0.59	137.53	0.979
	DM~PM+Sex+(1 Site)	5	-257.21	1.25	0.31	134.27	0.974
	DM~PM*Sex+(1 Site)	6	-254.85	3.62	0.10	134.38	0.974
	DM~PM+(1 Site)	4	-241.01	17.46	0.00	124.94	0.964
	DM~1+(1 Site)	3	-78.24	180.22	0.00	42.38	0.000

when scaling the relationship between FL and both FM and DM (Table 3; Fig. 4). Most LMMs including co-variables displayed comparable overall predictive ability as judged by their fitting scores (Table 4). In general, models accounting for sexual dimorphism

outperformed other models for all species, although in a few cases, parameters from equally supported baseline models (e.g., FL-only fixed-effect, Δ AICc < 2) led to more accurate body mass predictions (Table 4). Fixed effects were significant in all best-fitted models (in all cases $p > 0.001$, but $p = 0.048$ when predicting fresh mass for *B. nivalis*), but the random effect (i.e., site) was not significant for any model ($p > 0.001$; Suppl. material 1: Appendix 4). All formulated LMMs outperformed the null models (i.e., $\ln(\text{FM}) \sim 1 + (1|\text{Site})$ and $\ln(\text{DM}) \sim 1 + (1|\text{Site})$) in their predictive power.

We found that predicted body mass (both fresh and dry mass) was significantly correlated with empirical measurements; however, using PM as a predictor led to the most accurate estimates (Fig. 5). In all cases, the relationship between estimated and measured body mass was not significantly different from a 1:1 relationship, with > 89% of the variation explained (Table 5). The range of prediction error (RMSE) was near identical for body mass predictions obtained from mass-to-mass ratios, scaling regressions, and LMMs. When using PM as a predictor, FM estimates from PM:FM ratios were marginally more accurate than those from LMMs (RMSE = 0.011 g and 0.012 g, respectively). In contrast, LMMs were slightly more accurate than PM:DM ratios when predicting DM (RMSE = 0.014 g and 0.017 g, respectively). However, the range of prediction error was considerably higher when using FL as a predictor. For FM estimates, predictions based on SMA scaling relationships were marginally more accurate than those from LMMs (RMSE = 0.048 g and 0.050 g, respectively), but when predicting DM, prediction errors were identical using both methods (RMSE = 0.025 g).

Discussion

A key source of variation in morphological traits is measurement repeatability, which is inherently related to the statistical power of analyses based on those measurements (Bailey and Byrnes 1990, Wylde and Bonduriansky 2021). We found the highest repeatability for larger traits compared to smaller traits (e.g., femur length vs femur width), and the larger sex (female vs male) when pooling values for all size proxies and species. The effect of sex on repeatability was less clear when considering individual traits, suggesting that measurement repeatability in these species depends on other factors such as species size, trait size, and their interactions rather than sex alone. As noted by Bigelow (1967), measurement repeatability in these grasshoppers decreases in traits with rounded boundaries, such as femur width, and in traits where margins are highly variable in shape, such as pronotum length.

Table 3. Length–mass scaling coefficients for predicting both fresh and dry mass from femur length in three flightless New Zealand grasshopper species. Regression parameters based on standardized major axis regressions and their confidence intervals (95% CI) are shown.

Species	Model formulae	Intercept _(CI)	Slope _(CI)	R ²	p-value
(a) fresh mass (FM) as a function of femur length (FL)					
<i>Brachaspis nivalis</i>	$\ln(\text{FM}) \sim \ln(\text{FL})$	-7.754 _(-7.937, -7.571)	2.696 _(2.625, 2.768)	0.965	< 0.001
<i>Paprides nitidus</i>	$\ln(\text{FM}) \sim \ln(\text{FL})$	-8.914 _(-9.085, -8.743)	3.090 _(3.024, 3.157)	0.976	< 0.001
<i>Sigaia australis</i>	$\ln(\text{FM}) \sim \ln(\text{FL})$	-9.584 _(-9.783, -9.385)	3.315 _(3.241, 3.391)	0.982	< 0.001
(b) dry mass (DM) as a function of femur length (FL)					
<i>Brachaspis nivalis</i>	$\ln(\text{DM}) \sim \ln(\text{FL})$	-9.234 _(-9.747, -8.721)	2.778 _(2.585, 2.986)	0.898	< 0.001
<i>Paprides nitidus</i>	$\ln(\text{DM}) \sim \ln(\text{FL})$	-10.077 _(-10.504, -9.650)	3.071 _(2.908, 3.242)	0.953	< 0.001
<i>Sigaia australis</i>	$\ln(\text{DM}) \sim \ln(\text{FL})$	-11.423 _(-11.919, -10.927)	3.539 _(3.357, 3.731)	0.939	< 0.001

In addition, the orientation of specimens to the focal plane of the microscope can result in parallax error that is expected to be more pronounced for small structures that are difficult to measure (e.g., Wylde and Bonduriansky 2021). We found larger traits, such as femur length, could be measured with relatively little error compared to smaller features (femur width and pronotum length) that were subject to more parallax error. Measurement repeatability was also higher for the larger body mass measures (fresh and preserved mass) compared to dry specimens (dry mass), which had small values that were sensitive to variation in humidity. Dried

specimens become slightly hydrated during weighing, resulting in increased errors in measurement. Body length is a widely used linear metric, but we found it unreliable in grasshoppers, as abdomen size varied considerably with body condition, including reproductive state (Hochkirch and Gröning 2008, García-Navas et al. 2017). Furthermore, the extent of telescoping of abdominal segments (Bigelow 1967) and distortion during preservation are additional sources of measurement error (García-Barros 2015).

Collecting and storing insects in chemical fluids, such as ethanol, has the potential to alter their body mass (Moretti et al. 2017, Penell et al. 2018), thus limiting their use in ecological studies that require accurate body mass data (Leuven et al. 1985, Chown and Gaston 2010). We found that the weight loss of 95% ethanol-preserved specimens was largely restricted to the first two months of preservation after which weight stabilized, and only minimal differences were recorded (Suppl. material 1: Appendix 2). The high ethanol concentration (i.e., 95%) used here to also protect DNA could explain these results, as it would speed the leaching of water from tissues. Studies of aquatic insects show similar responses, with weight loss mostly limited to the first four weeks after preservation (e.g., Stanford 1973, Leuven et al. 1985, Cressa 1999, Wetzel et al. 2005). The degree of weight loss during preservation is a function of specimen size, which probably explains different responses to preservation of sexes. In absolute terms (g), larger specimens (females) lost more mass than smaller ones (males), yet the proportional difference (%) was negligible (Suppl.

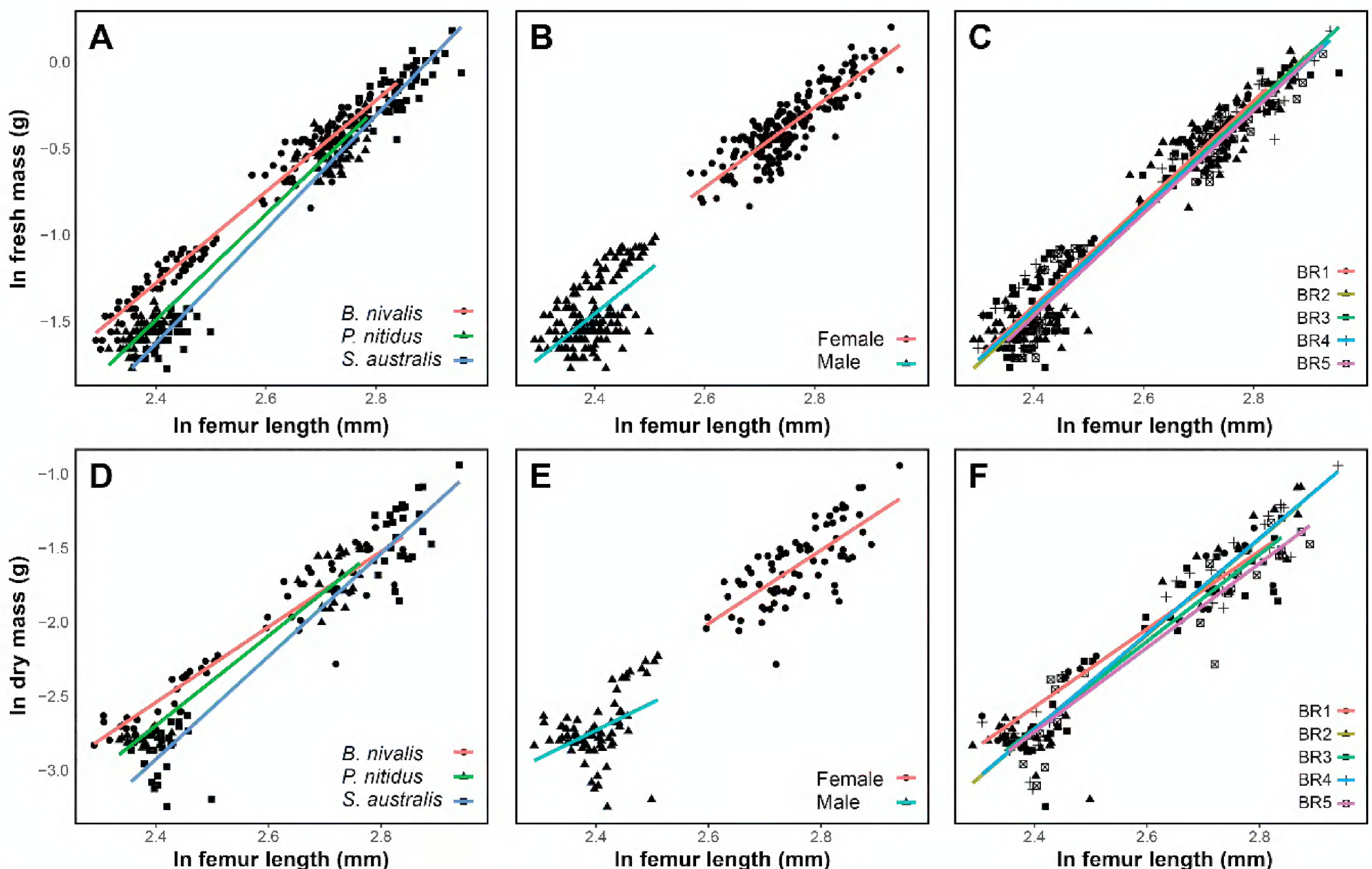


Fig. 4. Length-to-mass relationships in three flightless New Zealand grasshopper species showing the influence of elevation and sexual dimorphism. Fresh mass–femur length (A–C) and dry mass–femur length (D–F). Length–mass relationships are shown on natural logarithmic axes (ln). Sample sites (BR1 to BR5) indicating five sites in ~100-m elevation intervals from 1,383 to 1,817 m asl. Lines represent the best-fit from standardized major axis regressions. Credible intervals are omitted for clarity. Some regression lines overlaid each other.

Table 4. Model selection showing the best-fitted models (AICc in bold) for predicting both fresh mass and dry mass from femur length in three flightless New Zealand grasshopper species. Abbreviations: K = number of parameters, AICc = Akaike’s information criterion corrected for sample size, wi = Akaike weight, LL = Log-Likelihood, R² = marginal R². Model parameters of the best-fitting models (Δ AICc < 2) used for predictions are shown in Suppl. material 1: Appendix 4.

Species	Model formulae	K	AICc	Δ AICc	wi	LL	R ²
(a) fresh mass (FM) as a function of femur length (FL)							
<i>Brachaspis nivalis</i>	ln(FM)~ln(FL)*Sex+(1 Site)	6	-284.34	0.00	0.44	148.51	0.968
	ln(FM)~ln(FL)+Sex+(ln(FL) Site)	7	-283.44	0.90	0.28	149.18	0.969
	ln(FM)~ln(FL)+Sex+(1 Site)	5	-283.22	1.12	0.25	146.85	0.967
	ln(FM)~ln(FL)+(1 Site)	4	-278.56	5.78	0.02	143.44	0.965
	ln(FM)~1+(1 Site)	3	149.94	434.28	0.00	-71.88	0.111
<i>Paprides nitidus</i>	ln(FM)~ln(FL)+(1 Site)	4	-328.73	0.00	0.64	169.58	0.976
	ln(FM)~ln(FL)+Sex+(1 Site)	5	-327.15	1.58	0.29	169.88	0.981
	ln(FM)~ln(FL)*Sex+(1 Site)	6	-324.43	4.30	0.07	169.62	0.981
	ln(FM)~ln(FL)+Sex+(ln(FL) Site)	7	-296.49	32.24	0.00	152.38	0.981
	ln(FM)~1+(1 Site)	3	247.53	576.26	0.00	-120.68	0.000
<i>Sigauss australis</i>	ln(FM)~ln(FL)+Sex+(1 Site)	5	-180.21	0.00	0.65	95.46	0.987
	ln(FM)~ln(FL)+(1 Site)	4	-178.53	1.68	0.28	95.77	0.982
	ln(FM)~ln(FL)*Sex+(1 Site)	6	-175.85	4.36	0.07	95.61	0.987
	ln(FM)~ln(FL)+Sex+(ln(FL) Site)	7	-156.20	24.01	0.00	82.34	0.987
	ln(FM)~1+(1 Site)	3	204.43	384.63	0.00	-99.07	0.000
(b) dry mass (DM) as a function of femur length (FL)							
<i>Brachaspis nivalis</i>	ln(DM)~ln(FL)+(1 Site)	4	-45.22	0.00	0.48	27.05	0.904
	ln(DM)~ln(FL)+Sex+(1 Site)	5	-44.42	0.80	0.32	27.89	0.902
	ln(DM)~ln(FL)*Sex+(1 Site)	6	-43.14	2.08	0.17	28.55	0.904
	ln(DM)~ln(FL)+Sex+(FL Site)	7	-39.14	6.08	0.02	27.90	0.903
	ln(DM)~1+(1 Site)	3	66.45	111.67	0.00	-29.96	0.000
<i>Paprides nitidus</i>	ln(DM)~ln(FL)+Sex+(1 Site)	5	-69.02	0.00	0.53	40.21	0.962
	ln(DM)~ln(FL)*Sex+(1 Site)	6	-68.45	0.57	0.40	41.23	0.964
	ln(DM)~ln(FL)+(1 Site)	4	-67.97	1.05	0.03	35.94	0.955
	ln(DM)~ln(FL)+Sex+(ln(FL) Site)	7	-63.98	5.04	0.04	40.13	0.963
	ln(DM)~1+(1 Site)	3	85.46	154.48	0.00	-39.46	0.000
<i>Sigauss australis</i>	ln(DM)~ln(FL)+(1 Site)	4	-28.96	0.00	0.65	18.93	0.955
	ln(DM)~ln(FL)+Sex+(1 Site)	5	-26.75	2.21	0.21	19.06	0.955
	ln(DM)~ln(FL)+Sex+(ln(FL) Site)	7	-24.78	4.19	0.08	20.72	0.960
	ln(DM)~ln(FL)*Sex+(1 Site)	6	-24.27	4.70	0.06	19.11	0.956
	ln(DM)~1+(1 Site)	3	120.31	149.28	0.00	-56.90	0.000

material 1: Appendix 2). These results agree with previous studies (e.g., Wetzel et al. 2005, Paxton 2013); however, additional factors, such as surface area–volume ratio, environmental conditions, and concentration and volume of preservative, may also influence the leaching process (Leuven et al. 1985, Paxton 2013).

Studies of the mass-to-mass relationships of terrestrial insects are scarce (e.g., Edwards 1996, Penell et al. 2018). Here, we found that preserved mass was a prime predictor of body mass across all three grasshopper species, especially when predicting fresh mass (Tables 1, 2). Inter-individual differences during the drying process seemed to challenge model accuracy and fit when predicting dry mass. Visual inspection of dry mass–preserved mass regressions indicates unexplained size-related variance, meaning higher residual error in large individuals across all species. This suggests that inter-individual differences in body composition (e.g., water, carbohydrates, protein, and fat content) and condition (nutritional and reproductive status) may be important factors explaining such variance.

The choice of a robust linear size trait is an important consideration for accurate mass estimates when applying allometric scaling regressions (Gaston and Blackburn 2000, Moretti et al. 2017). Here, we showed that femur length strongly correlates with other body dimensions and body mass measures in all three grasshopper species, having by itself a high predictive power when estimating body mass at the species level, especially when predicting fresh mass (Tables 3, 4). Femur length has previously been shown to have a

linear relationship to body length in one of these species, which is in turn linearly related to body mass (Batcheler 1967). However, in all cases, body mass predictions based on preserved mass were substantially more accurate (Table 5), with prediction errors < 0.018 g (against < 0.050 g for predictions based on femur length). Thus, it seems sensible to use preserved mass as a predictor when basic knowledge of the effects of preservation method on body mass is available. Otherwise, femur length is the metric to be used, as more than 90% of the variance in body mass was described by this trait in all cases (Tables 3, 4). The addition of alternative body dimensions during the modeling process would result in marginal improvement of prediction accuracy but would substantially increase the time needed for processing samples (e.g., Sohlström et al. 2018).

As expected, sex was sometimes retained as an informative predictor of body mass when used in addition to or as an interaction with femur length. This is not surprising given that adult females of these grasshopper species are approximately three times as heavy as adult males. Including sex generally increased model fit (Tables 2, 4); however, its inclusion did not produce better estimates than traditional mass-to-mass and allometric scaling regressions (Table 5; Fig. 5). Similar allometric relationships have been found in bees and hoverflies (Kendall et al. 2019) where sex was influential in the fit of the models but not in their predictive power. Likewise, the use of sex-specific regressions did not produce better mass estimates than simple regressions for

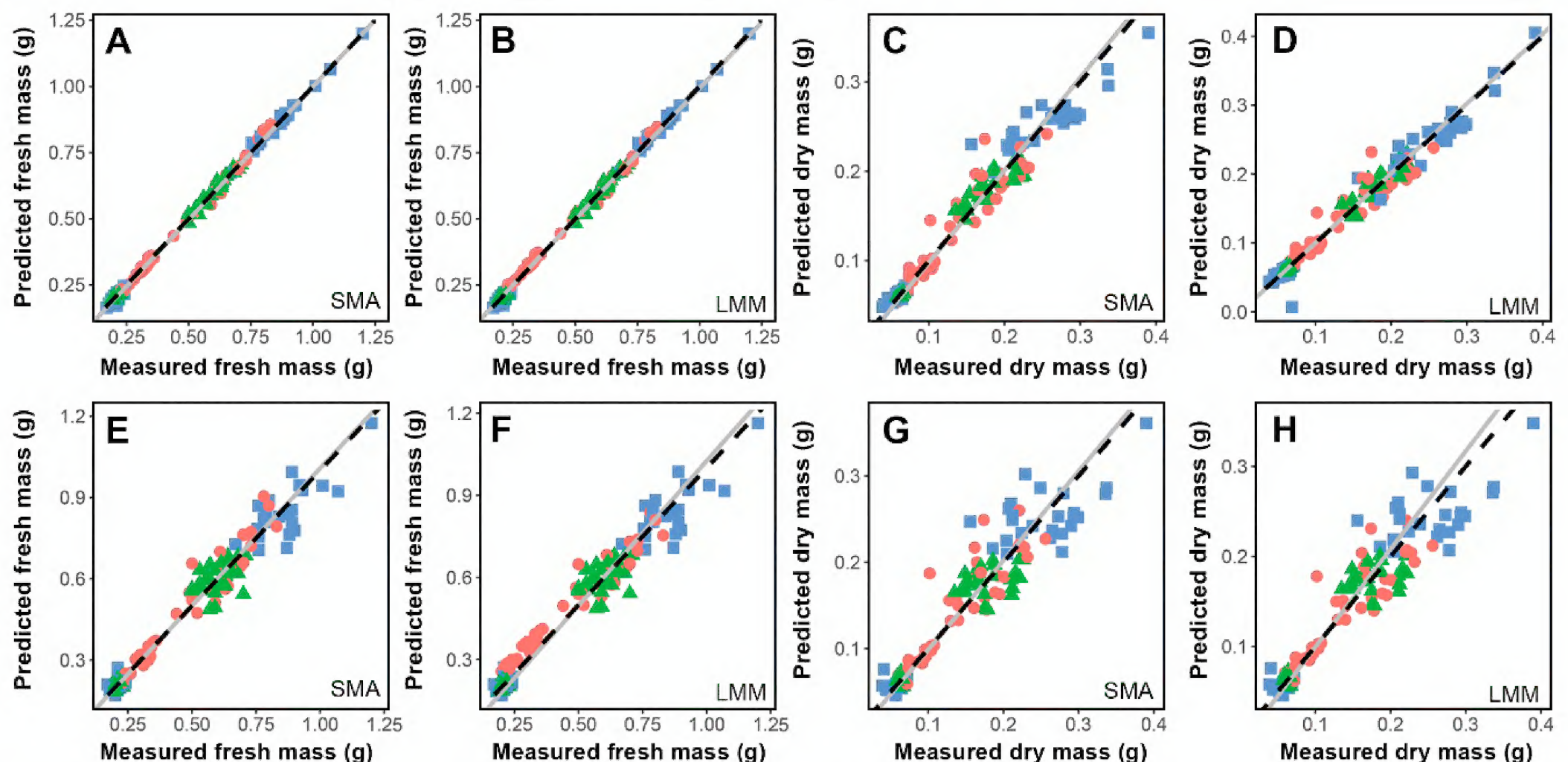


Fig. 5. High predictability observed when comparing measured and predicted body mass using type-II linear regression with a major axis approach. Predictions based on preserved mass (A–D) and femur length (E–H) pooling data from three flightless New Zealand grasshopper species: *Brachaspis nivalis* (pink circles), *Paprides nitidus* (green triangles), and *Sigaia australis* (blue squares). The expected $x = y$ relationship is shown in dashed black line, and the observed is shown in solid grey line. Predictions from standardized major axis regressions (SMA) and linear mixed-effects models (LMM) are shown. Note that in most cases fitting lines overlap.

European spiders (Penell et al. 2018). Although the use of models accounting for sexual size dimorphisms is desirable, it seems that, at least for our dataset, general regressions led to better estimates than more complex models. While femur length was an accurate predictor of body mass across species, it became less reliable when comparing sexes within a given species, which can be related to the fact that, contrary to body mass, femur length remains fixed throughout adult life. Adult body size variation in structural linear traits is affected by environmental factors during development (Davidowitz et al. 2004). Therefore, intraspecific changes in the average value of adult structural size traits will require changes in size and structure at the population level (Bailey et al. 2020).

The difficulty of accurately predicting intraspecific body size variation based on co-varying linear traits is not new. The lack of predictive power has previously been explained in terms of traits varying in response to environmental conditions during development (Hagen and Dupont 2013, Kendall et al. 2019). Thus, decomposing a multidimensional trait, such as body size, into linear measures seems insufficient to capture intraspecific body size variation. Indeed, body size in the broadest sense is closely linked to the volume of an organism, which in linear terms is described by length, width, and height (Moretti et al. 2017, Sohlström et al. 2018). With this in mind, predictions based on models incorporating complementary morphological traits directly related to width and/or height (e.g., femur length in addition to pronotum width) would improve intraspecific body mass prediction accuracy, and thus the applicability of allometric scaling for exploring the ecological implications of widespread phenomena, such as sexual size dimorphism. Given that the sexes commonly respond differently to environmental shifts, a considerable amount of the unexplained intraspecific variation observed here may be related to sexual differences in body size plasticity (e.g., Stillwell et al. 2010).

Table 5. Details of statistical models (type II linear regression with a major axis) testing the relationships between predicted and measured body mass in three flightless New Zealand grasshopper species. Predictions are based on mass-to-mass ratios and scaling parameters from standardized major axis regressions (SMA) and linear mixed-effects models (LMM). The R^2 values, estimated intercept, and slope (95% confidence intervals) are given.

Model	Sample size	R^2	Intercept _(CI)	Slope _(CI)	p-value
(a) Preserved mass to fresh mass					
PM:FM ratio	368	0.998	0.004 _(0.002–0.006)	0.993 _(0.989–0.998)	< 0.001
LMM	368	0.998	-0.004 _(-0.006–0.002)	1.005 _(1.004–1.010)	< 0.001
(b) Preserved mass to dry mass					
PM:DM ratio	150	0.957	-0.006 _(-0.011–0.001)	1.035 _(1.000–1.071)	< 0.001
LMM	150	0.958	-0.004 _(-0.009–0.001)	1.032 _(0.997–1.068)	< 0.001
(c) femur length to fresh mass					
SMA	368	0.965	-0.006 _(-0.021–0.009)	1.013 _(0.982–1.045)	< 0.001
LMM	368	0.963	-0.031 _(-0.048–0.016)	1.056 _(1.023–1.091)	< 0.001
(d) femur length to dry mass					
SMA	150	0.898	-0.004 _(-0.013–0.002)	1.034 _(0.979–1.092)	< 0.001
LMM	150	0.901	-0.009 _(-0.018–0.002)	1.088 _(1.031–1.148)	< 0.001

The slope parameter β (power coefficient) of our femur length regressions ranged between 2.152 and 3.293 for fresh mass and between 2.544 and 3.425 for dry mass, thus being close to 3 as expected for animals with isometric growth (Suter and Stratton 2011). These values are higher than those from pre-existing allometric models (Schoener 1980) for tropical orthopterans ($\beta = 1.65–1.96$ for dry mass estimates), further supporting differences in slopes between insects from different climatic zones: tropical insects usually have smaller gradients than temperate ones (Schoener 1980). Interestingly, the slopes of temperate grasshoppers from North America

(Sabo et al. 2002) are around the lower limit of those reported here for New Zealand grasshoppers ($\beta = 2.274$ for dry mass estimates). However, regression parameters for North American grasshoppers were obtained using body length as a co-variable, thus preventing reliable comparison, as allometric relationships often differ between traits. As noted above, the use of body length in allometric studies on grasshoppers is not recommended, as this trait is difficult to measure accurately, thus jeopardizing model predictive power. Therefore, we expect our regressions to be highly applicable to ecological studies on New Zealand grasshoppers.

One source of potential error in our models is intraspecific regional variation in body size. This limitation can be problematic because scaling relationships in terrestrial insects, and thus, their regression parameters, are likely to vary geographically if populations' body size evolve independently of one another depending on local conditions (e.g., Johnston and Cunjak 1999, Sohlström et al. 2018, Kendall et al. 2019). New Zealand grasshoppers exhibit variations in body size among populations inhabiting elevational and latitudinal gradients (Bigelow 1967, Staples 1967, Mason 1970). By including size data from specimens collected on an elevational gradient in our models, we expect to have improved model robustness and reduced, at least in part, the effects of geographic size variation on their predictive power (Figs 3, 4). Variation in response to sampling season is expected to represent an additional source of error in our models, as average body size can change from year to year at the same site due to differences in environmental conditions (Bigelow 1967). Therefore, the performance of our models could be affected when predicting mass estimates from individuals with size measures far outside the trait ranges reported here.

Ecological applications.—Here we show that, for New Zealand grasshoppers, two easy-to-measure, non-destructive, and highly repeatable size estimates (i.e., preserved mass and femur length) are good predictors of other difficult-to-measure but ecologically meaningful size traits, such as fresh and dry mass. Many ecological disciplines typically require body mass data to relate body size to a range of ecological attributes. For example, body mass has been proposed as a suitable metric for testing ecogeographic patterns, such as Bergmann's rule (Blackburn et al. 1999). Since body mass is universally comparable, it is the metric of choice in macroecological studies interested in body size variation or size-dependent ecological processes (Gaston and Blackburn 2000). Body size estimates for New Zealand grasshoppers are more frequently available as body size dimensions (but see Batcheler 1967), making mass-to-mass and length-mass regression models useful for increasing our ability to further explore the ecological implications of body size.

Body mass estimates from scaling regressions have proven useful for studying aspects shaping arthropod communities including biomass production (e.g., Eklöf et al. 2017, Penell et al. 2018, Kinsella et al. 2020) and abundance (White et al. 2007). Traditionally, size-abundance relationships rely on fresh body mass of organisms (White et al. 2007, Sohlström et al. 2018), which is not available for most species. Thus, mass estimates from scaling regressions will alleviate this limitation. Given the apparent linear relationship between body size and consumption rate in the grasshopper species we examined (White 1975), indirect body mass estimates from length-mass regressions could also be used to predict herbivore impact on plant communities. For example, grasshopper dry mass correlates negatively with plant biomass in the field (Moretti et al. 2013), providing a potential trait for predicting plant consumption (Deraison et al. 2014).

Recently, declines in body size have been proposed as a general response to anthropogenic climate change in both endothermic and ectothermic animals (Gardner et al. 2011). Examining trends in body size requires the use of consistent size measures, and unfortunately, data often come as different size proxies, thereby hindering comparisons (Bailey et al. 2020). Body mass estimates from scaling regressions have helped to overcome this limitation by providing a tool for making size metrics from different sources (e.g., museum specimens, published datasets, and fresh sampling) comparable, so that tests of body size responses to climate change and warming temperatures can be performed (e.g., Tseng et al. 2018). This approach has proven useful for studying the trends and drivers of the change in the biomass of flying insects over time and space (e.g., Macgregor et al. 2019, Kinsella et al. 2020) and can now be used to estimate the body mass of New Zealand grasshoppers from historical abundance datasets (e.g., White 1975, White and Sedcole 1991).

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Supplementary material 1

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Data type: docx file

Explanation note: **Appendix 1.** Measurement repeatability based on repeated measures of body size traits from the same specimens in three New Zealand grasshopper species. **Appendix 2.** Effect of the preservation method (i.e., 95% ethanol) on body mass by comparing mass estimates between live (fresh mass) and preserved states (preserved mass after two and four months of preservation) in three New Zealand grasshopper species. **Appendix 3.** Intraspecific relationships between preserved mass and both fresh and dry mass in three New Zealand grasshopper species. **Appendix 4.** Regression parameters for the best-fitted linear mixed-effect models for body mass prediction based on preserved mass (Table 2) and femur length (Table 4) in three New Zealand grasshopper species. **Appendix 5.** Relationships between mass estimates (g) and body dimensions (mm) in three New Zealand grasshopper species. **Appendix 6.** Non-linear models fitted to describe intraspecific allometric relationships between mass estimates (FM and DM) and femur length (FL) in three New Zealand grasshopper species.

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